BOILED BONE GRAFT

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CITATION:

Emoto, Shuji. BOILED BONE GRAFT. 日本外科宝函 1956, 25(2): 115-137

ISSUE DATE:

1956-03-01

URL:

http://hdl.handle.net/2433/206255

日本外科寶函 第25卷 第2号

ARCHIV FÜR JAPANISCHE CHIRURGIE

XXV. BAND, 2. HEFT, 1. MÄRZ, 1956.

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BOILED BONE GRAFT

by

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[Received for Publication : Jun. 31. 1956]

INTRODUCTION

In modern brain surgery, the fresh auto-bone-graft or the bank-bone is routinely used for repair of a skull defect.[1] In extraordinary cases, however, one can use boiled skull flaps.[2][3][4][5][6][7] The skull flap is boiled, because the flap has accidentally been contaminated in the operating room, or because the flap is infiltrated by tumour tissue. In the majority of these cases, the healing of the boiled flap appears successful. In some cases, the fate of the graft has been traced by means of roentgenogram. However, there is no histological evidence as to whether the graft shows fibrous or osseous healing. As long ago as 1893, BARTH[8] found in animal experiments, that the boiled skull grafts were replaced by new bone tissue, and he called this healing process by the name "creeping substitution". After BARTH no experiments of the boiled skull graft seem to have been reported in literature. There are surely not a few experimental studies on the fate of the boiled long bone graft, but the discrepancy of the results is so pronounced that the detailed research on this problem is now advisable. The purpose of the present experiments is to study the fate of the boiled bone piece transplanted in skull or in fibula, as compared with the fate of the fresh bone piece transplanted similarly.

EXPERIMENTAL PROCEDURE

1. Experimental Animal.

Young mongrel dogs, weighing 3～5kg.

2. Graft Bone and Graft Bed.

Under ether anesthesia, the parieto-temporal pericranium was incised and ablated.

And four holes were made with a hand drill on each side of the skull. And then the bone was cut by a chisel from one hole to the other. With this procedure, each one piece of graft bone was subperiosteally taken from each side. Skull defects remaining after the graft bones had been taken off, were used as graft beds, into which the following bone grafts were placed; (1) autogenous or homogenous graft bone which had previously been boiled in physiological saline solution for ten minutes, and (2) autogenous or homogenous fresh bone. And then the cut lines of pericranium were sutured as close as possible. At the same time or after a few days, transplantation experiments of fibula were made in the following way. Fibula was exposed on each side and periosteum was cut longitudinally with a knife and ablated from the shaft. And then a part of the shaft, 1.3 cm long, was resected subperiosteally. The resected bone was treated in the same manner as in case of skull, and placed into the defect of the shaft like a bridge. After this transplantation, the cut line of the periosteum was closed by suture. Thus one dog had four kinds of graft. Against hemorrhage from the skull and infection, "bone wax" and penicillin were used respectively. Modes of transplantation in eleven dogs were shown in Table 1.

Table 1

	left side boiled	right side fresh	cases histologically examined after one year	cases histologically examined after a few weeks or months
1st type experiment	homograft	autograft	2	1
2nd type experiment	homograft	homograft	2	1
3rd type experiment	autograft	autograft	2	3

3. Macroscopical, Roentgenographical and Histological Examinations.
One year after transplantation, dogs were killed by means of intravenous injection of formalin and then the skull and the fibula containing the graft bone were taken out and examined macroscopically and roentgenographically. These specimens were fixed with formalin and decalcified in trichlor-acetate solution in the lower atmospheric pressure. Celloidin technic was used and specimens were stained by hematoxylin-eosin and by VAN GIESON method.

DETAILS OF RESULTS

A. Observation after One Year.

No. 16 Skull on the 379th day and fibula on the 376th day after transplantation. Fresh autograft on right side and boiled homograft on left (Fig. 1~8).

16SL (skull, left). Boiled homograft (left side) on the 379th day. Roentgenographical findings: Drill holes and cut lines are clearly recognized. Graft is a little absorbed. Microscopical findings: Graft; Nowhere callus is seen. Gaps between the graft- and host-bone are filled with connective tissue. Pericranium has normal appearance and shows no evidence of osteogenic activity. Some marrow cavities contain fibrous tissue, while others have fat. In haversian canals vessels and connective tissue are present. Around haversian canals and marrow cavities there are revitalized lamellae, though in the remaining parts of the graft lacunae

are entirely vacant and look like desert, which has nothing alive. Host-bone; In some small parts near the cut ends, lacunae are empty, and marrow cavities are fibrous, but in other parts the bone tissue has normal appearance.

16SR (skull, right). Fresh autograft (right side) on the 379th day. Roentgenographical findings : Drill holes and cut lines are recognized. Graft is a little absorbed. Microscopical findings : Graft ; Nowhere osteogenic activity is recognizable. Gaps between the graft- and host-bone are filled with connective tissue. Pericranium is thin. Near the pericranium there is a silk thread knot surrounded by connective tissue and by a small collection of leucocytes. Meninx becomes somewhat thicker, but shows no sign of osteogenesis. Some marrow cavities have fat, and others have connective tissues. Haversian canals are of normal appearance. Though small dead parts are scattered here and there, the larger parts have normal lamellae and osteocytes. Host-bone ; Near the cut ends there is a very small dead part. In other parts, the host-bone shows entirely normal appearance.

16FL (fibula, left). Boiled homograft (left side) on the 376th day. Roentgenographical findings: Cut lines are not detectable. Fibula appears normal. Microscopical findings: Osteogenic activity is not seen. Any trace of the graft can not be detected. But with accurate observation, we can find some dead parts of bone, in which vacant haversian canals run. Probably they would be the ruins of the old boiled graft.

16FR (fibula, right). Fresh autograft (right side) on the 376th day. Roentgenographical and microscopical findings: Entirely the same as above (16FL), but the dead parts are smaller.

No. 6 Skull on the 360th day and fibula on the 352nd day after transplantation. Fresh autograft on right side and boiled homograft on left.

6SL (skull, left). Boiled homograft (left side) on the 360th day. Roentgenographical findings: Three drill holes and two cut lines are evident, but remaining one drill hole and two cut lines are not visible. Microscopical findings : Graft ; In a gap between the graft- and host-bone, there are three "bone wax" clots. Another gap is not recognizable. Pericranium has normal appearance. Meninx is taken off. Content of marrow cavities is normal. Osteocytes are present throughout. But in a few small parts, osteocytes are lost. Haversian canals are normal. Connective tissue is seen invading into a marrow cavity. Host-bone ; There are no dead parts and no marrow cavities, which have abnormal contents. In a word, the boiled graft has undergone such a profound bony transformation, that if a gap and small dead parts were not found, I might mis-believe that I had an error in cutting out the specimen.

6SR (skull, right). Fresh autograft (right side) on the 360th day. Roentgenographical findings: One cut line is recognizable. Other cut lines and drill holes cannot be recognized. No sign of absorption of the graft. Microscopical findings : Graft; Gaps between the graft- and host-bone are filled with connective tissue. Osteocytes are present throughout. Very small areas are present, in which lacunae

are vacant. Marrow cavities and haversian canals have normal content.

6FL (fibula, left). Boiled homograft (left side) on the 352nd day. Roentgenographical findings: Cut lines are not visible. The fibula has normal configuration. Microscopical findings: Periosteum, arrangement of haversian canals and osteocytes are almost normal, but with accurate observation small parts are found, in which lacunae are vacant.

6FR (fibula, right). Fresh autograft (right side) on the 352nd day. Roentgenographical findings: Cut lines are not visible. Fibula has normal configuration. Microscopical findings: Periosteum and arrangement of haversian canals are normal. In a small area between periosteum and cortical surface a crowd of fibroblast-like cells and some osteoclasts are present, and the cortex is eroded at this area.

No. 13 Skull and fibula on the 378th day after transplantation. All homografts, boiled on left side and fresh on right.

13SL (skull, left). Boiled homograft (left side) on the 378th day. Roentgenographical findings: Drill holes and cut lines are indistinct, but the graft is considerably absorbed. Microscopical findings: Graft; One of the gaps remains open and is occupied by fibrous tissue. Nearly a half of the graft is absorbed, and small osseous islets are scattered. The other end of the graft has united with the host-bone. Pericranial and meningeal osteogenic processes are not in progress. Marrow cavities have fibrous tissue. Host-bone; Haversian canals are of normal appearance. Near one of the cut ends, there are a few small parts in which osteocytes are absent.

13SR (skull, right). Fresh homograft (right side) on the 378th day. Roentgenographical findings: Four drill holes are recognizable, but cut lines are not seen. Graft is not absorbed. Microscopical findings: Graft; Gaps between the graft- and host-bone are not visible. Nowhere osteogenic activity. Marrow cavities and haversian canals have normal appearance. Osteocytes are normal.

13FL (fibula, left). Boiled homograft (left side) on the 378th day. Roentgenographical findings: Cut lines are not visible. Microscopical findings: Gaps between the graft- and host-bone cannot be detected. There are remnants of silk threads in the bone. There are considerably large dead parts of the bone. Haversian canals which run through the dead areas are very indistinct.

13FR (fibula, right). Fresh homograft (right side) on the 378th day. Roentgenographical findings: Cut lines are not seen. Fibula shows normal form. Periosteum, haversian canals and osteocytes are entirely normal. Accurate observation reveals small parts, in which lacunae are vacant.

No. 3 Skull on the 373rd day and fibula on the 369th day after transplantation. All homografts, boiled on left side and fresh on right.

3SL (skull, left). Boiled homograft (left side) on the 373rd day. Roentgenographical findings: Drill holes and cut lines are indistinct, but a large part of the graft is absorbed. Microscopical findings: Outside the graft, there is an intense infiltration of leucocytes. In the center of this infiltration, three silk thread knots are found. Graft is very thin. Gaps between the graft- and host-bone are filled

with connective tissue. In nearly all microscopic fields, osteocytes are clearly stained. But in some parts, lacunae are vacant. Haversian canals are enlarged. On the walls of these canals, osteoblasts stand side by side, i. e. osteogenic process is going on. Host-bone ; Pericranial or meningeal osteogenic activity is not seen. Marrow cavities and haversian canals have normal appearance, but near the cut ends haversian canals are enlarged. Some marrow cavities have "bone wax".

3SR (skull, right). Fresh homograft (right side) on the 373rd day. Roentgenographical findings : Two drill holes still remain. The other drill holes and all cut lines cannot be observed. Graft is not absorbed. Microscopical findings : Graft ; Graft- and host-bone are connected with fibrous tissue. Pericranium shows no osteogenic activity. Majority of marrow cavities are occupied by fat, but a few of them are fibrous. Some haversian canals are enlarged, and on the walls of these canals osteoblasts stand side by side. Osteocytes are present throughout the graft, but in some parts there are empty lacunae. Host-bone ; Near the cut ends, a few haversian canals are enlarged and osteoblasts are recognized.

3FL (fibula, left). Boiled homograft (left side) on the 369th day. Roentgenographical findings : Cut lines are not seen, but fibula becomes thicker to a small extent. Microscopical findings : Fibula is thickened. Osseous union between the graft- and host-bone is perfect. Considerably large dead areas are scattered. Some haversian canals are enlarged and fibrous.

3FR (fibula, right). Fresh homograft (right side) on the 369th day. Roentgenographical findings : Cut lines are not seen. Fibula is of normal form. Microscopical findings : Osseous union between the graft- and host-bone is perfect. Periosteum and osteocytes have normal appearance. Almost all haversian canals have normal content, but some of them are enlarged and invaded by fibrous tissue. Marrow cavities contain fat.

No. 20 Skull on the 357th day and fibula on the 352nd day after transplantation. All autografts, boiled on left side and fresh on right.

20SL (skull, left). Boiled autograft (left side) on the 357th day. Roentgenographical findings : Drill holes and cut lines are not distinct. Graft is partly absorbed. Microscopical findings : Osseous union between the graft- and host-bone. Osteogenic process is nowhere going on. Majority of marrow cavities contain fat, but some contain fibrous tissue. Haversian canals are normal. In small parts, lacunae are empty.

20SR (skull, right). Fresh autograft (right side) on the 357th day. Roentgenographical findings : Drill holes and cut lines are indistinct. Absorption of the graft is not evident. Microscopical findings : Osseous union between the graft- and host-bone is perfect. We cannot see the border of the graft. Osteogenic activity is not recognized. Majority of marrow cavities have fat, but a few have fibrous tissue. There are small necrotic areas.

20FL (fibula, left). Boiled autograft (left side) on the 352nd day. Roentgenographical findings : Fibula has entirely normal form (Fig. 11). Microscopical findings : Some small dead areas are recognized. Majority of haversian canals are

normal and osteocytes have normal appearance.

20FR (fibula, right). Fresh autograft (right side) on the 352nd day. Roentgenographical findings: Fibula appears entirely normal. Microscopical findings: Arrangement of haversian canals is normal and osteocytes have entirely normal appearance. Marrow cavities are normal.

No. 14 Skull and fibula on the 378th day after transplantation. All autografts, boiled on left side and fresh on right.

14SL (skull, left). Boiled autograft (left side) on the 378th day. Roentgenographical findings: One drill hole is clear, but the other holes and cut lines are indistinct. The graft is a little absorbed. Microscopical findings: Graft; the graft-bone is thinner than the host-bone, but osseous union is so complete that we can not see the border of the graft. Osteogenic process is not going on. There are large parts, in which lacunae are empty. Haversian canals have normal appearance. Appositional bone from the meninx is evident. There are bony islands in the meninx.

14SR (skull, right). Fresh autograft (right side) on the 378th day. Roentgenographical findings: Drill holes and cut lines are indistinct. The graft is partially absorbed. Microscopical findings: One of the two gaps seen in the preparation between the graft- and host-bone is filled with fibrous tissue, and another with osseous tissue. Pericranium and meninx show no evidence of ostcogenic activity. The graft-bone is thinner. Some of the marrow cavities are fibrous, but others are normal. Haversian canals show normal appearance. Very small dead foci are present in the graft. Appositional bone from the meninx is evident. Host-bone; Near the cut ends there are small areas, in which lacunae are empty. The host-bone is very thick and nearly a half of its thickness would have been produced probably from meninx.

14FL (fibula, left). Boiled autograft (left side) on the 378th day. Roentgenographical findings: The original position of the graft is not evident. Fibula has normal form. Microscopical findings: There is some deformity at the site of transplantation. But osseous union is so perfect that we can not see the border of the graft. Arrangement of haversian canals and of osteocytes is irregular at the junction of the graft. Considerably large dead areas are present.

14FR (fibula, right). Fresh autograft (right side) on the 378th day. Roentgenological findings: Fibula has entirely normal appearance. Microscopical findings: There are very small necrotic foci. In larger parts, periosteum, haversian canals and osteocytes are entirely normal.

B. Observation after a Few Weeks or Months.

No. 5 Skull on the 45th day and fibula on the 34th day after transplantation. Fresh autograft on right side and boiled homograft on left.

5SL (skull, left). Boiled homograft (left side) on the 45th day. Roentgenographical findings: Cut lines and drill holes are clearly recognizable. The graft is somewhat absorbed. Microscopical findings: Graft; Approximation is good. The

graft- and host-bone are connected with newly formed bone tissue. Pericranium is not thick. Outer plate of the graft is partially eroded and diploe is widely destructed. A few bony islands are present, around which osteogenic activity is going on. Osteocytes in the graft are lost. Some haversian canals are fibrous. Host-bone; Near the cut ends, there are some necrotic areas. Marrow is intact. To the periosteal surface newly formed bones are attached. Also the meninx on the inner surface of the host-bone is the source of new bones.

5SR (skull, right). Fresh autograft (right side) on the 45th day. Roentgenographical findings: Drill holes are recognizable, but cut lines are somewhat indistinct. Microscopical findings: Graft; Approximation is very good. One of the gaps is united by newly formed bone, but another one is filled with connective tissue. From meninx new bone is formed and covers the inner surface of the graft. Osteocytes of the transplanted bone are entirely lost. Haversian canals contain living cell elements. Majority of the marrow cavities are filled with fibrous tissue, but some cavities are normal. In about one quater of the graft "creeping substitution" has taken place. From pericranium comes scanty new bone to the outer surface of the graft. Host-bone; A few small necrotic areas are present near the cut ends. The meninx becomes very thick, in which newly formed bony islands are scattered.

5FL (fibula, left). Boiled homograft (left side) on the 34th day. Roentgenographical findings: Graft-bone has no callus around it, but host-bone has callus near the cut ends. Microscopical finding: Graft; Graft is surrounded by a crowd of leucocytes and a fibrous membrane. Osteocytes are lost. All haversian canals contain leucocytes. The central part of the graft is eroded by a crowd of leucocyte-like cells. Near one end, large cancellous bones are newly formed. Host-bone; One end of the host-bone is eroded by leucocyte-like cells. Inner layers of the cortex have vacant lacunae. Large callous masses are attached to the host-bone. Marrow cavities have normal content.

5FR (fibula, right). Fresh autograft (right side) on the 34th day. Roentgenographical findings: Graft- and host-bone are connected by osseous tissue. Microscopical findings: Graft; About two-thirds of the graft are absorbed. All osteocytes disappear, except in the superficial layer of the cortex. Newly formed lamellae are found on the walls of enlarged haversian canals. Into the marrow cavities invades fibrous tissue. The graft is surrounded by enormous newly formed cancellous bones. They contain cartilage tissue, in which enchondral ossification is taking place. In gaps between the graft- and host-bone, cancellous bone and cartilage tissue are scattered. Host-bone; Marrow cavities are normal. No osteocytes are changed. Around the host-bone there are cancellous bones.

No. 4 Skull on the 37th day and fibula on the 19th day after transplantation. All homogenous grafts, boiled on left side and fresh on right(Fig. 13～20).

4SL (skull, left). Boiled homograft (left side) on the 37th day. Roentgenographical findings: Cut lines and drill holes are recognizable, but edges of some holes are not distinct. Microscopical findings: Graft; Adaptation is good. Union

by cancellous bone has been made between the graft- and host-bone. Osteogenic activity of the pericranium is not seen, but the dura is thickened and forms new bone surrounding the graft. Osteocytes of the graft disappear and lacunae are empty. In marrow cavities, there is fibrous tissue, but no marrow cell. Almost all haversian canals have living cell elements. Creeping substitution is in progress. Host-bone; Osteogenic activity of the pericranium is not recognized. On the contrary, meningeal osteogenic process is seen near the cut edge to the extent of about one centimeter. Dead parts are present near the cut edges.

4SR (skull, right). Fresh homograft (right side) on the 37th day. Roentgenographical findings: Cut lines except one are clearly seen. Drill holes are not distinct. Graft is not absorbed. Microscopical findings: Graft; Approximation is good. Pericranium is not thick. Erosions of the bone are present under pericranium, where many osteoclasts collect. Heavy thickening of meninx is recognized and dense connective tissue proliferates therefrom in and around the graft. Nearly a half of the graft is destructed, and absorbed. All osteocytes are lost. Haversian canals contain connective tissue and vessels. Host-bone; In small parts osteocytes disappear. Osteogenic activity of the pericranium is not seen. On the contrary, a considerable amount of cancellous bone is attached to the inner surface of the skull.

4FL (fibula, left). Boiled homograft (left side) on the 19th day. Roentgenographical findings: Approximation is good. Around the graft some callus is formed. The surrounding bone becomes thicker. Graft is not absorbed. Microscopical findings: Graft; Approximation is good. Gaps between the host-bone and graft are filled with connective tissue and osseous union has not occurred. Lacunae are empty and haversian canals contain leucocyte-like cells. The marrow cavities are also occupied by leucocyte-like cells and dead cells. The graft is surrounded immediately by fibrous tissue, and outside of it by a large callus. Beneath the periosteum there is cartilage tissue, at the edges of which enchondral ossification proceeds. Host-bone; Around the host-bone, abundant cancellous bone develops. In larger parts of the host-bone osteocytes are normal. Marrow cavities of the host-bone are also normal.

4FR (fibula, right). Fresh, homograft (right side) on the 19th day. Roentgenographical findings: Spicula-formation is demonstrated between the host-bone and fragment. Around the graft- and host-bone, distinct callus develops. Microscopical findings: Graft; Nearly one half of the graft is destructed and all osteocytes are absorbed. Some haversian canals contain connective tissue and leucocytes. And along the canals erosion of bone proceeds. Around the destructed graft, there is a massive callus, in the center of which cartilage tissue is observed. Numerous osteoclasts are also present. Host-bone; Massive cancellous bones have developed around the host-bone. Osteocytes are not absorbed. Haversian canals and marrow cavities are of normal appearance.

No. 15 Skull and fibula on the 7th day after transplantation. All autografts, boiled on left side and fresh on right.

15SL (skull, left). Boiled autograft (left side) on the 7th day. Roentgenographical findings: Drill holes and cut lines are distinct. Graft is not absorbed. Microscopical findings: Graft; Approximation is good. Gaps between the graft- and host-bone are filled with connective tissue. The pericranium is attached closely to the surface of the graft and in some parts shows some osteogenic activity. Meninx is taken off. In the marrow cavities no element is alive, except at the open ends. Haversian canals contain living cell elements. Osteocytes are recognizable in all lacunae, but they are poorly stained. They have disappeared in some areas near the cut ends. Host-bone; Pericranium shows no osteogenic activity. On the contrary meninx becomes thicker and forms new bone surrounding the host-bone. Osteocytes are seen in all lacunae except near the cut end. Marrow cavities have normal appearances.

15SR (skull, right). Fresh autograft (right side) on the 7th day. Roentgenographical findings: Drill holes and cut lines are clearly recognizable. Absorption of the graft has not begun. Microscopical findings: Graft; Adaptation is good. Gaps between the graft- and host-bone are filled with dense connective tissue. Osteogenic activity of pericranium is not seen. On the contrary, meninx becomes thicker and forms cancellous new bone around the graft. Marrow cavities have dense fibrous tissue. Some haversian canals contain living elements, while others contain dead elements. Osteocytes are present near the surfaces, but in the deeper layer all osteocytes are lost. Host-bone; Both meninx and pericranium form massive cancellous bones on each surface of the host-bone. Osteocytes are present, but lacunae near the cut ends are vacant.

15FL (fibula, left). Boiled autograft (left side) on the 7th day. Roentgenographical findings: Adaptation is good. Cut lines are distinct. Absorption of the graft is not seen. Microscopical findings: Graft; Gaps between the graft- and host-bone are filled with connective tissue. Osteocytes are recognizable in all microscopic fields, but somewhat poorly stained by haematoxylin. The marrow cavities contain dead elements. No evidence of absorption of the graft. Haversian canals have also dead elements. Host-bone; Periosteal osteogenic activity is seen. Callus radiates from the surface of the host-bone. One islet of cartilage tissue is present near the periosteum.

15FR (fibula, right). Fresh autograft (right side) on the 7th day. Roentgenographical findings: Adaptation is good at one end of the graft, but bad at another end. Microscopical findings: Graft; Gaps between the host-bone and graft are filled with fibrous tissue. Periosteal osteogenic activity is intense and newly formed cancellous bones surround the graft. In outer layers of these new bones there is cartilage tissue. The marrow cavities are normal. Some haversian canals have living elements, but others have dead ones. Majority of lacunae are empty. The enlargement of haversian canals is distinct. Host-bone; Newly formed bone surrounds the graft. Osteocytes are recognizable in all microscopic fields except those near the cut ends. The marrow cavities are normal.

No. 2 Skull and fibula on the 12th day after transplantation. All autograft,

boiled on left side and fresh on right.

2SL (skull, left). Boiled, autograft (left side) on the 12th day. Roentgenological findings: Drill holes are clearly seen, but cut lines are not distinct. Absorption of the graft is not apparent. Microscopical findings: Graft; Approximation is good. In the gaps between the graft- and host-bone, there are many new bones. Pericranium and meninx show no osteogenic activity. There is dead tissue in marrow cavities, into which connective tissue is invading from the open ends. Haversian canals have nothing alive. In the gaps between the host-bone and graft, some blue-stained homogenous masses ("bone wax") are present. Osteocytes are recognizable in nearly a half of the microscopic fields, but they are poorly stained. Host-bone; In some part near the cut ends the host-bone has vacant lacunae. Around the host-bone, there is no osteogenetic activity. Contents of marrow cavities are normal.

2SR (skull, right). Fresh, autograft (right side) on the 12th day. Roentgenographical findings: Drill holes are distinct, but cut lines are not so distinct. Absorption of the graft is not apparent. Microscopical findings: Graft; Approximation is not good. Osteocytes are recognizable in a few parts, but missing in larger parts. Pericranium and meninx do not show any osteogenic activity. Contents of haversian canals and marrow cavities are all living cells. Host-bone; There are some dead areas near the cut ends. Meninx forms massive callus, but pericranium does not. Clots of "bone wax" are surrounded by fibrous tissue.

2FL (fibula, left). Boiled, autograft (left side) on the 12th day. Roentgenographical findings: Approximation is good. Callus is not visible. Absorption of the graft is not apparent. Microscopical findings: Osteocytes are recognizable in all lacunae, but poorly stained. Haversian canals have a few leucocytes. Contents of marrow cavities are dead. Osteogenic activity is not seen. Host-bone; There are small dead parts in the host-bone, which is surrounded by newly produced cancellous bones.

2FR (fibula, right). Fresh, autograft (right side) on the 12th day. Roentgenographical findings: One cut line is visible, but others are indistinct. Absorption of the graft is not apparent. Microscopical findings: Graft; In the superficial layer of the cortex osteocytes are seen, but in the inner layers they have disappeared. Haversian canals have elements alive. Fibrous tissue invades into the marrow cavities. The graft is encapsulated by loose connective tissue. Osteogenic activity is not evident. Host-bone; Near the cut ends, there are some dead parts. Marrow cavities are of normal appearance. Newly formed cancellous bone are attached to the surface of the host-bone.

No. 1 Skull on the 17th day and fibula on the 12th day after transplantation. All autografts, boiled on left side and fresh on right.

1SL (skull, left). Boiled autograft (left side) on the 17th day. Roentgenographical findings: Drill holes and cut lines are recognizable, but indistinct. Graft is not absorbed. Microscopical findings: Graft; Approximation is good. In the middle of the graft runs a fracture line. Two gaps are filled with connective

tissue, and one of them has a slender bridge of new bone. Osteogenic activity of the pericranium is absent. On the contrary, meninx forms new bone which proliferates onto the surface of the graft. Into the marrow cavities of the graft, connective tissue invades, but "creeping substitution" has not been completed. Osteocytes are recognizable in all microscopic fields, but poorly stained. Host-bone; Abundant callus is attached to the bone surface. In some small parts near the cut ends, osteocytes are lost. Marrow cavities and haversian canals have normal contents.

1SR (skull, right). Fresh autograft (right side) on the 17th day. Roentgenological findings: Drill holes are recognizable but somewhat indistinct. Cut lines are vague. Microscopical findings (Fig. 21, 22): Adaptation is very good. Graft and host-bone are connected with new bone. Pericranium does not evidently produce bone, though the surface of the graft is a little eroded. On the contrary, meninx thickens and forms new bones which proliferate onto the surface of the graft. There is no cartilage tissue. Connective tissue invades into marrow cavities. Nearly all haversian canals have elements alive. In the superficial layer of the skull osteocytes are seen, but in other parts they have disappeared. In some of the marrow cavities, "creeping substitution" is going on. Host-bone; Pericranium and meninx produce abundant new bone around the graft. Cavities in new bone trabeculae are filled with loose connective tissue, looking like bone marrows. Osteocytes near the cut ends are lost. Marrow cavities and haversian canals have normal contents.

1FL (fibula, left). Boiled autograft (left side) on the 12th day. Roentgenographical findings: Adaptation is bad. Callus is not formed around the graft-bone. A small quantity of callus is found around the surface of the host-bone. Microscopical findings: Graft; Periosteum does not show osteogenic activity. The surface of the graft is not eroded. Osteocytes are visible, but stained poorly. Contents of the marrow cavities and haversian canals are dead. Host-bone; Periosteum forms callus. Osteocytes disappear in small parts near the cut ends, where the bone is eroded and replaced by dense fibrous tisse.

1FR (fibula, right). Fresh autograft (right side) on the 12th day. Roentgenological findings: Adaptation is good. Callus is present. Graft is not yet absorbed. Microscopical findings: Graft; Gaps between the host-bone and graft are filled with connective tissue. Periosteum forms cancellous bone which is deposited on the surface of the graft. The surface of the graft and haversian canals are eroded and partially replaced by dense connective tissue. Contents of marrow cavities are homogeneous and dead. Some osteocytes remain in the superficial layer of the graft. Host-bone; Around the surface of the host-bone, there are large cancellous bones. On the outside of this bone, there is cartilage tissue. Near the end of the host-bone, some osteocytes are lost. Marrow cavities have normal content.

COMMENT

1. *The mode of the healing of a boiled bone graft.* BARTH stated that the

graft bone once died out and then was replaced by newly formed bone from the host bed. This is a principle acceptable with some exceptions in transplantation of bone in general. A number of experiments and clinical experiences in this line have been reported.[9~13] Recently CAMPBELL[14] proved this principle by experiments using various bones. Also my experiments show that not only boiled bone grafts, but also fresh ones heal though the process of substitution of bone tissue.

2. *Is there any difference in the final outcome between boiled and fresh bone grafts, and also between auto- and homografts?* It is generally accepted that a fresh autograft is the best material for transplantation, and a boiled bone is less suitable. However, I can not find any experimental work in literature, in which the fate of a boiled bone graft was followed up and compared with that of a fresh bone graft in the course of one year. This has been done in my experiments. The results of my long-term observation show some difference between fresh and boiled grafts, but little between homo- and autografts. In general, the bony substitution of a boiled bone graft is poorer than that of a fresh bone graft (Fig. 1 ~8). This difference is observed more markedly in the skull than in the fibula. In addition, two of the skull grafts in my experiments underwent mild infection and were partially lost by absorption, but largely revitalized. Thus a boiled bone graft may be of practical use, as YAMAKI[15] formerly stated.

3. *Does the observation during one year show the final result?* It is said that, in animal experiments, it takes for the complete transformation of a graft bone a period of time from three months to one year after transplantation.[16][17] In this experiment it has been evidenced that after one year absorption and regeneration of bone are no longer actively going on, i. e. the reparation of bone is in the stationary states. Thus it may be supposed that the dead parts in our specimens will no further be repaired.

4. *Does osseous union between the graft- and host-bone take place?* Fluoroscopically perfect osseous union is uniformly observed in every fibula (Fig. 9, 12). The skull, on the contrary, sometimes shows osseous union, but other times does not. It seems that occurrence of osseous union is related not to the kind of the graft bone used, but rather to the individuality of an experimental animal. Thus the osseous union is observed almost equally in each pair of roentgenograms of the skull (Fig. 10). It is well known that in man fracture lines, drill holes and sawing lines of the skull remain open for a long time. Some surgeons consider that osseous union of the skull never occurs in humans.[18] According to EGGERS' experiment,[19] skull flaps of rats never heal by osseous union without being compressed continually against the edge of the host-bone. In my experiments, however, osseous union has occurred frequently and intensely.

5. *Is the rate, with which a graft bone is incorporated, different according to the kind of the graft done?* Various graft bones are incorporated almost in the same manner, but the rate of the incorporation is not the same.[14][20][21][22] This fact is clearly observed in a series of roentgenograms of fibula which have been taken every month (Fig. 11). The boiled graft bone is definitely slower in process of

incorporation than the fresh graft bone.

6. *Disappearance and reappearance of osteocytes in the boiled graft.* After boiling, the bone tissue preserves the stainability so well, that microscopically it is not easy to differentiate the boiled bone from the fresh one. However when a boiled bone is transplanted into a host, osteocytes are stained gradually worse, though identifiable up to 12 days after transplantation. On the contrary, osteocytes of a fresh graft are absorbed more rapidly and lacunae become empty as early as on the 7th day after transplantation. In other words osteocytes of a boiled bone graft disappear more slowly than those of a fresh bone graft. This fact has already been noticed by PHEMISTER,[17] who states that dead osteocytes are liquefied by ferment and absorbed, and when a graft bone is boiled, the protein of cells is coagulated by heating, and becomes more resistible to ferment. I also found that the absorption of a fresh graft bone took place earlier and more conspicuously than that of a boiled bone (Fig. 13~20).

The absorption of a graft bone progresses along the surface, haversian canals and marrow cavities, and the new formation of bone follows along them. This phenomenon is a typical creeping substitution. As the result of observations in the course of one year, it is concluded that the absorption and replacement of a fresh graft bone are not only more rapid but also more exhaustive than those of a boiled bone, and that therefore a fresh bone graft is more favorable for transplantation. Nowadays a refrigerated bone graft is widely used in Europe and America. The purpose of this procedure is not to keep the cells of the graft bone alive, but to maintain their chemico-physical property "fresh".[23] Reverse is the ORELL's idea of "Os purum".[24] "Os purum" is a bone which is deprived of all organic substances by the action of chemicals and heat, in order to be easily invaded by tissue elements of the host. But the absorption of "Os purum" seems to be delayed.

7. *Appearance of cartilage tissue in the newly formed bone.* No cartilage tissue is seen in specimens after one year. But in earlier stages after transplantation, cartilage tissue is observed in fibula, (Fig. 17~20), but not in skull (Fig. 21, 22). PHEMISTER[17] and BLOCK[25] reported the same fact. Embryologically the bone of cranial vault is produced by means of intramembranous ossification (namely direct transformation of fibrous tissue into osseous). On the contrary, long bones are produced by means of enchondral ossification. Accordingly the appearance of cartilage tissue in fibula and non-appearance in skull perhaps mean the difference of embryological development.

8. *Attitude of host-bone.* In early stages after transplantation, there are found near the cut edges considerably large areas, in which osteocytes disappear and lacunae are empty. Later, "creeping substitution" takes place in some of these areas. Even after one year the host-bone contains some dead parts near the cut edges of skull. This phenomenon has been recognized by PHEMISTER[17] and REYNOLDS,[26] and suggests that osteocytes are so sensitive to injury that even in a fresh autograft osteocytes once die out, being finally replaced by regenerated ones.

9. *Has meninx osteogenic ability?* BLOCK[25] states that pericranium, endosteum

and meninx have osteogenic ability, and amongst these three the most active one is meninx. In early stage after transplantation in my experiment, large network-like new bones are produced by meninx and attached to the surfaces of the graft- and host-bone. (Fig. 21, 22). This fact evidently indicates the osteogenic ability of meninx. And at the same time, more or less pectinate new bones are produced by pericranium. Thus osteogenic process takes place probably both in pericranium and meninx.

10. "*Bone wax*". "Bone wax" clot is not absorbed even after one year and is surrounded by fibrous tissue, as is silk thread, showing fibrous incorporation. "Bone wax" may be regarded as a relatively inert substance. Because of the fact, that no cells can invade "bone wax" clot, a mass of wax used at operation must interfere with the union between the graft- and host-bone.

11. *Can we expect the good healing of a boiled bone graft in humans* ?
As compared with the experiments of other researchers,[27][29] the present experiments have shown the very good incorporation of a boiled graft. Although I do not know the true reason why such excellent results have been obtained, following conditions may have to be taken into consideration: (1) younger dogs were used, (2) skull flaps were smaller as compared with those in case of human craniotomy, and (3) periosteum of fibula was always kept intact.

SUMMARY

In dogs I have made some experiments of bone transplantation. The fate of a boiled bone graft has been compared with that of a fresh bone graft in fibula or in skull. The results of the experiments are summarized as follows.

1) Both boiled and fresh bone grafts show osseous incorporation in fibula and to a certain degree in skull one year after transplantation.

2) Fibula (after one year) : In both boiled and fresh bone grafts osseous union between the graft- and host-bone is so complete that even the roentgenogram can not reveal the grafted bone. However microscopically some dead parts are remaining which are larger in a boiled bone graft than in a fresh bone graft.

3) Skull (after one year) : Osseous union between the graft- and host-bone is not complete so that the site of transplantation can be easily recognized by roentgenogram. In a fresh bone graft revitalized parts are large and dead parts are small, being scattered like islets in the graft. In a boiled bone graft revitalized parts are smaller.

4) One year after transplantation, osteogenic process is no longer actively going on in both fibula and skull. This fact shows that the transformation of bone has already stopped.

5) Transformation of a fresh bone graft begins and stops earlier than that of a boiled bone graft.

6) Osteocytes of all bone grafts disappear in early stage. And osteocytes of a boiled bone graft disappear slower than those of a fresh bone graft.

7) In the course of bony transformation, cartilage tissue appears in fibula, but never in skull.

8) Not only meninx but also pericranium have the osteogenic ability.

9) "Bone wax" is not absorbed, but remains there surrounded by fibrous tissue.

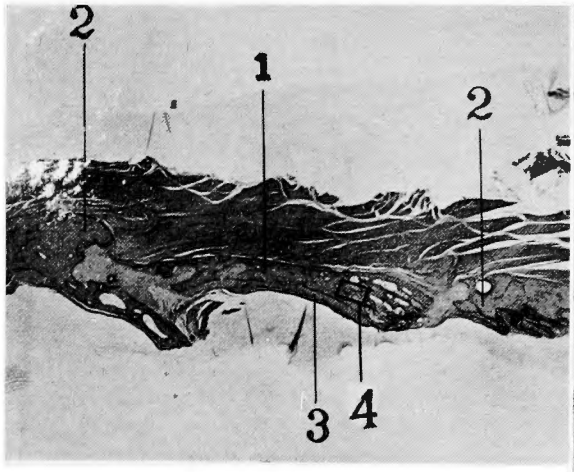

Fig. 1 Experiment No. 16. Skull, boiled homograft. 379 days after transplantation. (1) Graft-bone. (2) Host-bone. (3) Meninx. (4) Enlarged in Fig. 2. There is no osseous union between the graft- and host-bone.
Fig. 1-8 : the same animal.

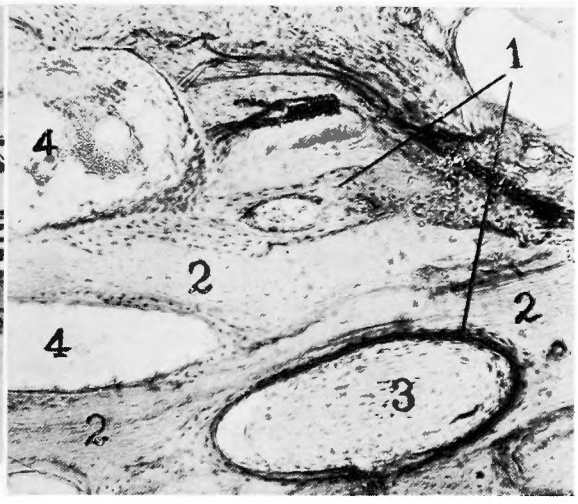

Fig. 2 A part of the graft-bone (4 in Fig. 1) is enlarged (× 55). (1) Viable osteocytes ar present around the margins of haversian canals and marrow cavities. (2) Old bone in which lacunae are empty. (3) Marrow cavity contains fibrous tissue. (4) fat marrow.

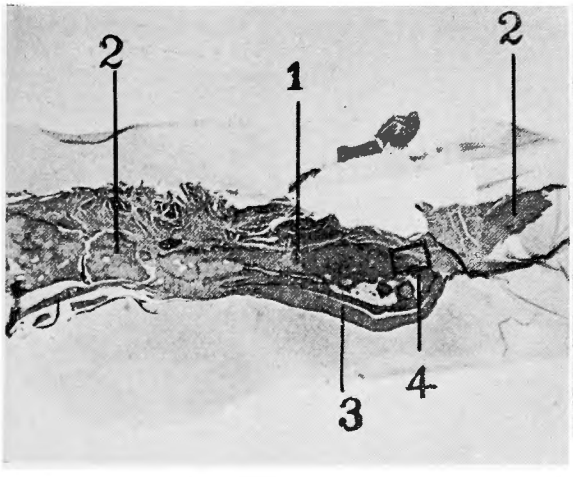

Fig. 3 Experiment No. 16. Skull, fresh autograft. 379 days after transplantation. (1) Graft-bone. (2) Host-bone. (3) Meninx. (4) Enlarged in Fig. 4. There is no osseous union between the graft-and host-bone.

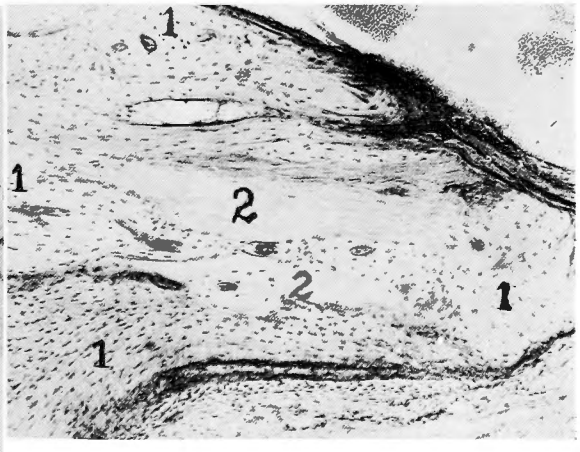

Fig. 4 A part of the graft bone (4 in Fig. 3) is enlarged (× 55). (1) Viable osteocytes. (2) Remnants of the old graft-bone are seen like islets.

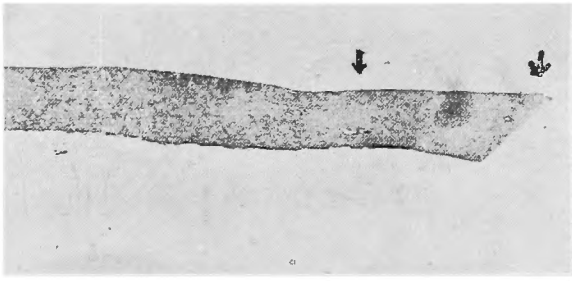

Fig. 5 Experiment No. 16. Fibula, boiled homograft. 376 days after transplantation. Osseous union between the graft- and host-bone is complete so that the fibula shows the normal configuration.

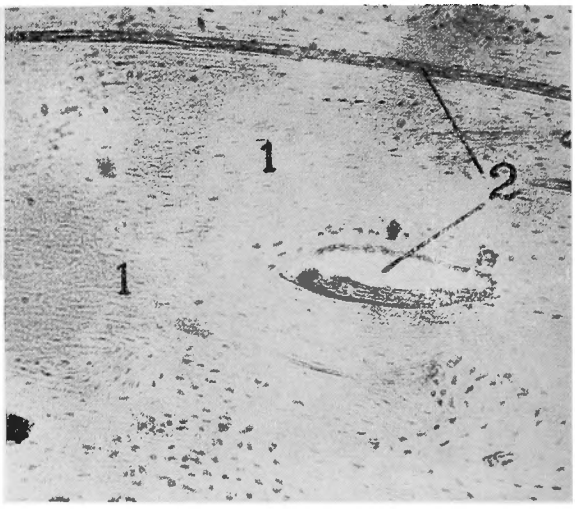

Fig. 6 A part of fibula (between the two arrows in Fig. 5) is enlarged. (1) There is a considerably large part, in which lacunae are empty. (2) Viable osteocytes are present around haversian canals.

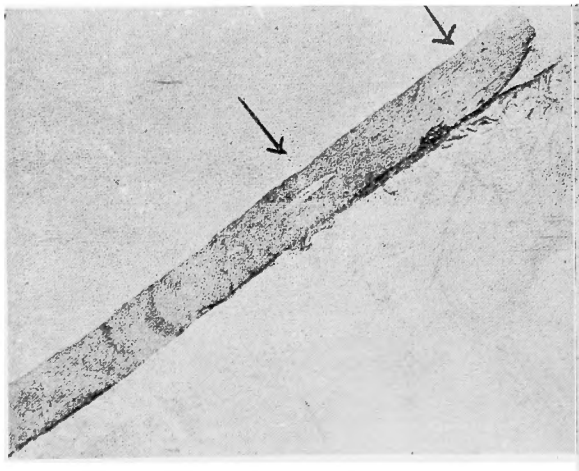

Fig. 7 Experiment No. 16. Fibula, fresh autograft. 376 days after transplantation. Osseous union between the graft- and host-bone is complete. Fibula shows the normal configuration.

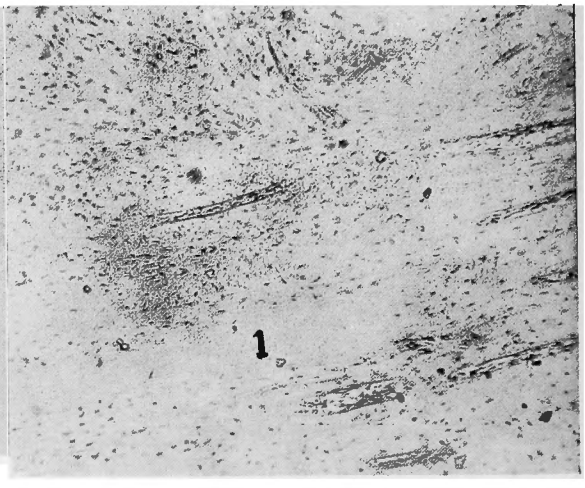

Fig. 8 A part of the graft between the two arrows in Fig. 7) is enlarged (× 55). (1) Here is a small part, in which lacunae are empty. In other parts, viable osteocytes are seen.

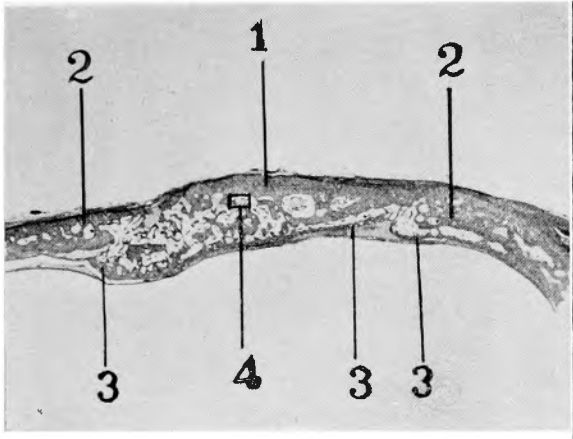

Fig. 13 Experiment No. 4. Skull, boiled homograft. 37 days after transplantation. (1) Graft-bone. (2) Host-bone. (3) New bone above meninx. (4) Enlarged in Fig. 14.
Fig. 13 – 20 : the same animal.

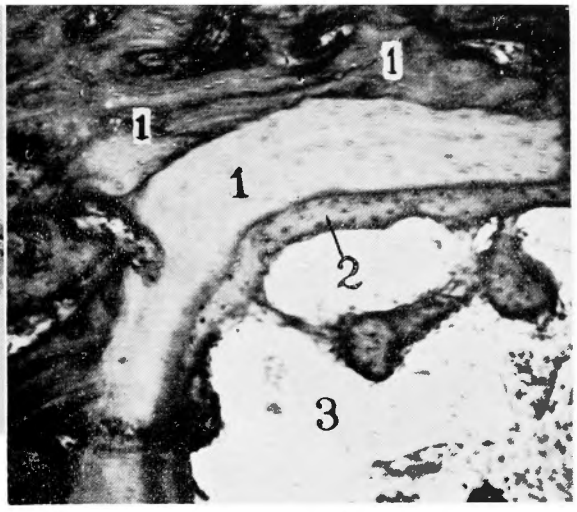

Fig. 14 A part of Fig. 13 (4) is enlarged (× 150). (1) Graft bone in which osteocytes are absorbed. (2) Newly formed lamellae in the wall of a marrow cavity. (3) Marrow cavity.

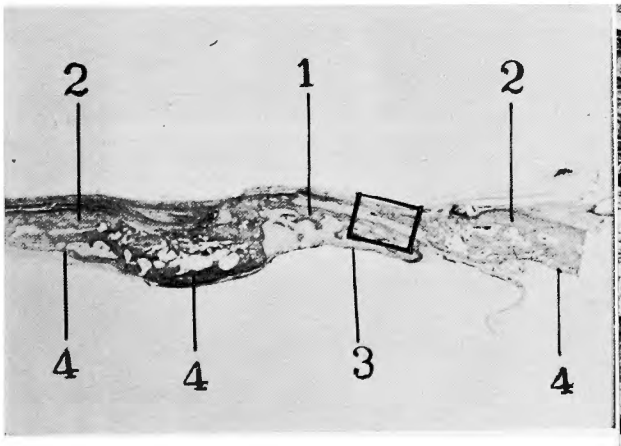

Fig. 15 Experiment No. 4. Skull, fresh homograft. 37 days after transplantation. (1) Graft-bone is heavily absorbed. (2) Host-bone. (3) Meninx. (4) New bone above meninx.

Fig. 16 A part of Fig. 15 (rectangle) is enlarged. (1) Graft-bone is heavily absorbed. (2) Pericranium. (3) Meninx. (4) Osteoclasts. →

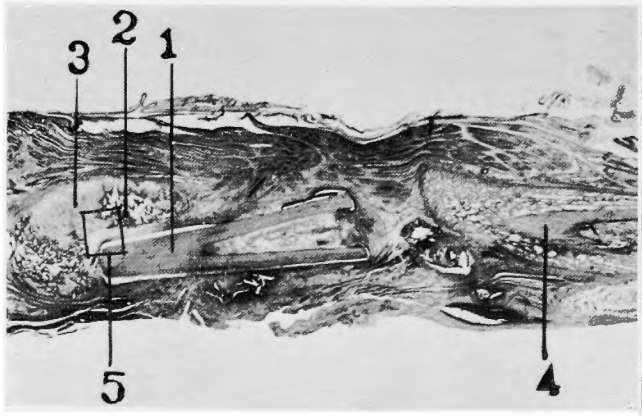

Fig. 17 Experiment No. 4. Fibula, boiled homograft. 19 days after transplantation. (1) Graft-bone. (2) Callus. (3) Cartilage. (4) Host-bone. (5) Enlarged in Fig. 18.

Fig. 18 A part of Fig. 17 (5) is enlarged (×55). (1) One end of the graft-bone. (2) New bone formed by enchondral ossification. (3) Cartilage tissue. →

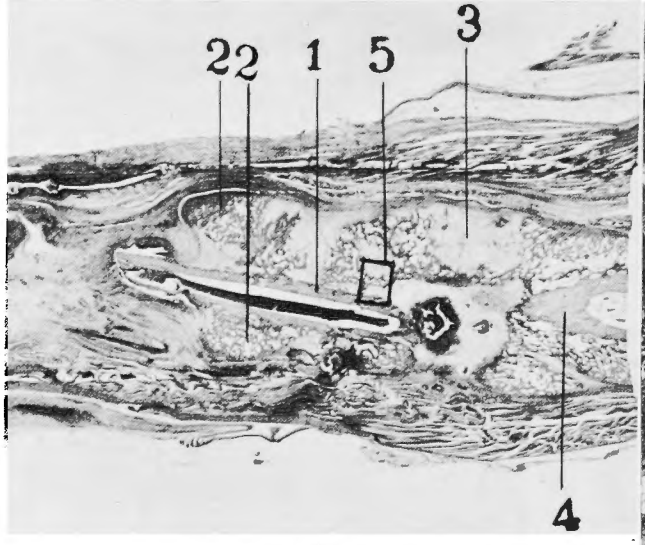

Fig. 19 Experiment No. 4. Fibula, fresh homograft. 19 days after transplantation. (1) Graft-bone is heavily absorbed. (2) Callus. (3) Cartilage tissue. (4) Host-bone. (5) Enlarged in Fig. 20.

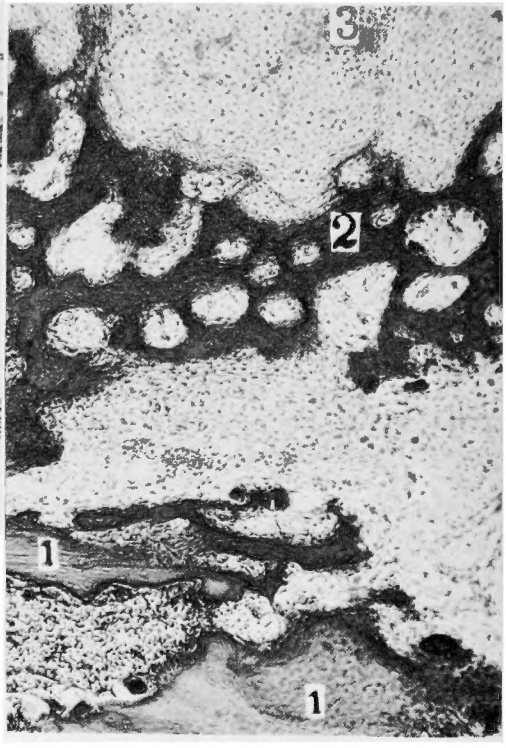

Fig. 20 A part in Fig. 19 (5) is enlarged (×55). (1) Graft-bone is heavily absorbed. (2) New bone formed by enchondral ossification. (3) Cartilage tissue. →

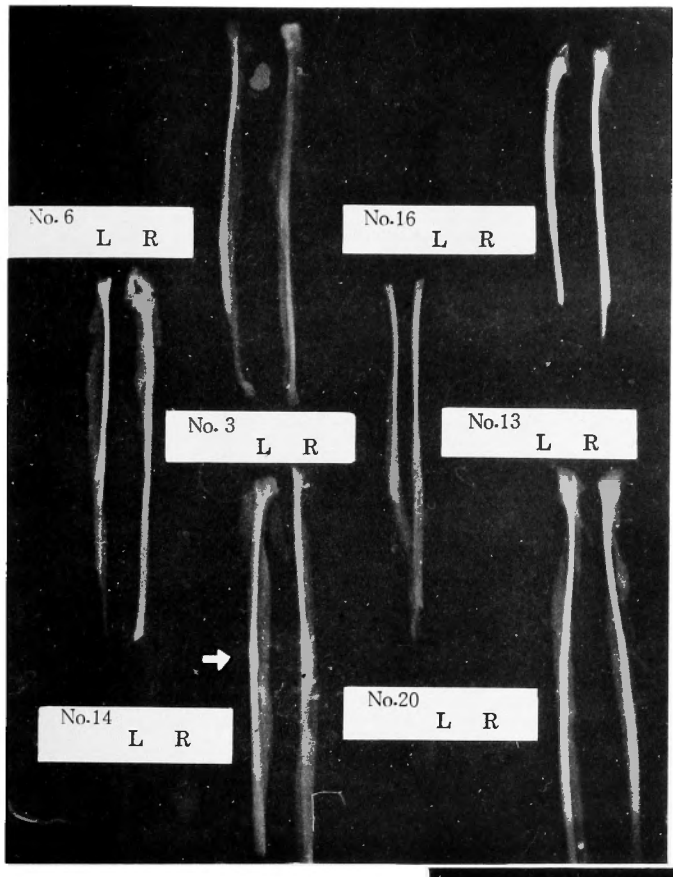
No. 6
L R
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Fig. 9

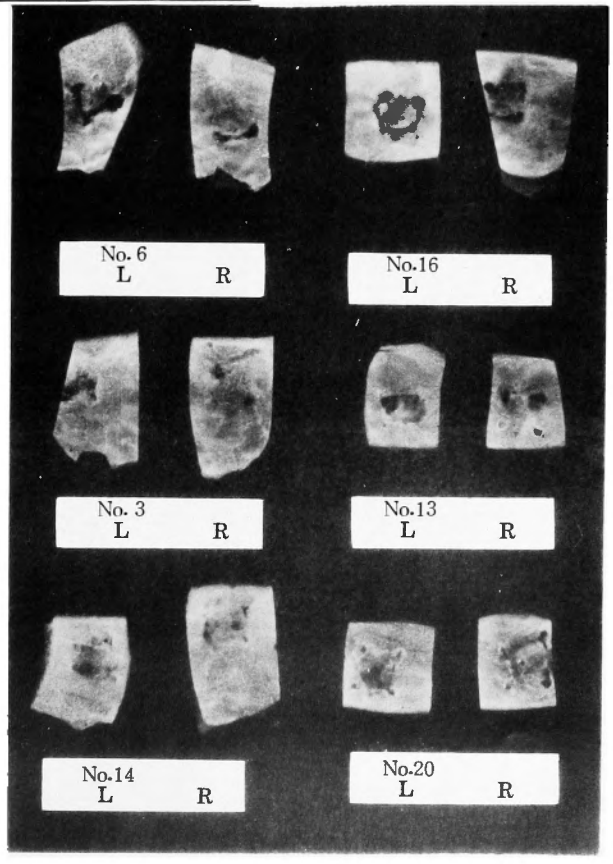
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Fig. 10

Fig. 9 Roentgenograms of removed fibulas. Nearly one year after transplantation.

	L(left side)	R(right side)
upper row	boiled homograft	fresh autograft
middle row	boiled homograft	fresh homograft
lower row	boiled autograft	fresh autograft

All fibulas appear nearly normal.

Fig. 10 Roentgenograms of excised skulls. Nearly one year after transplantation.

	L(left side)	R(right side)
upper row	boiled homograft	fresh autograft
middle row	boiled homograft	fresh homograft
lower row	boiled autograft	fresh autograft

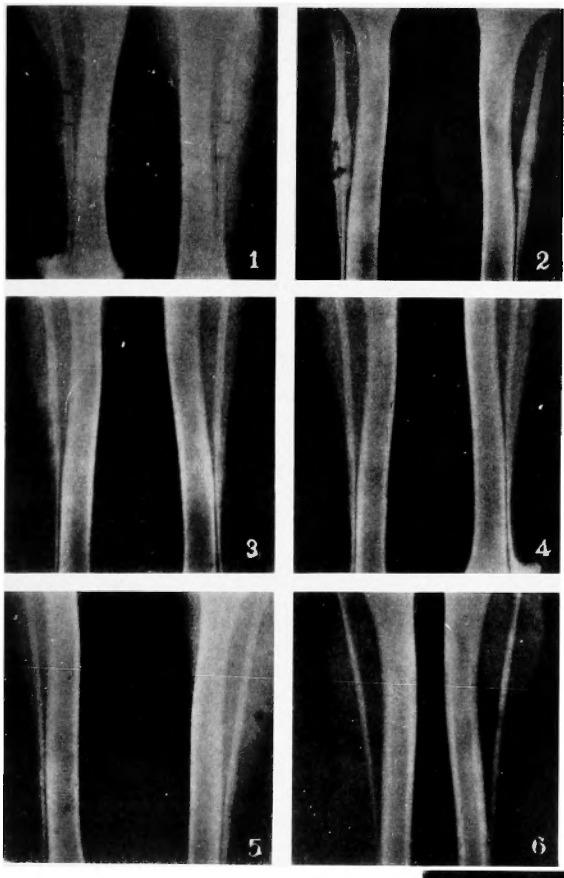

Fig. 11

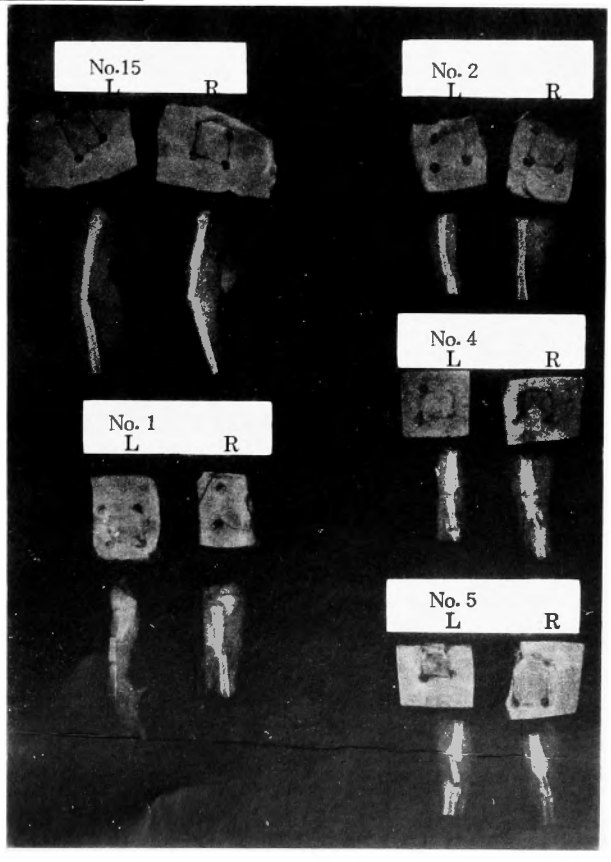

Fig. 12

Fig. 11 Experiment No. 20. Roentgenographical tracings of grafted fibulas. Left side, boiled autograft, and right side, fresh autograft. (1) Immediately, (2) 30days, (3) 73 days, (4) 100 days, (5) 120days, (6) 352 days after transplantation respectively.

Fig. 12 Roentgenograms of excised skulls and removed fibulas. L(left side) boiled, R(right side) fresh graft.

					left	right
No. 15	Sk. 7	days	Fib. 7	days	Autogr.	Autogr.
No. 1	Sk. 17	days	Fib. 12	days	Autogr.	Autogr.
No. 2	Sk. 12	days	Fib. 12	days	Autogr.	Autogr.
No. 4	Sk. 37	days	Fib. 19	days	Homogr.	Homogr.
No. 5	Sk. 45	days	Fib. 34	days	Homogr.	Autogr.

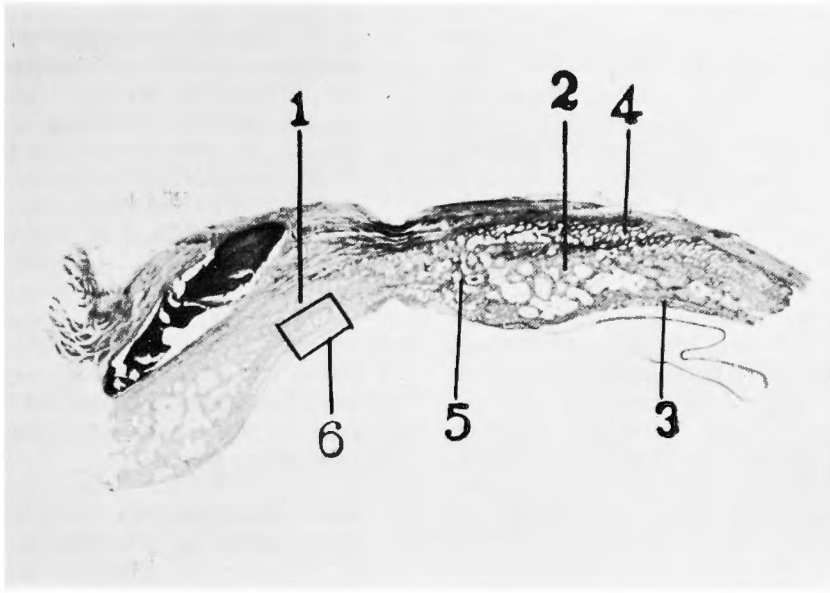

Fig. 21 Experiment No. 1. Skull, fresh autograft. 17 days after transplantation. (1) Graft-bone. (2) Host-bone. (3) New bone between meninx and host bone. (4) Pectinate new bone from pericranium. (5) New bone connecting the graft- and host-bone. (6) Enlarged in Fig. 22.

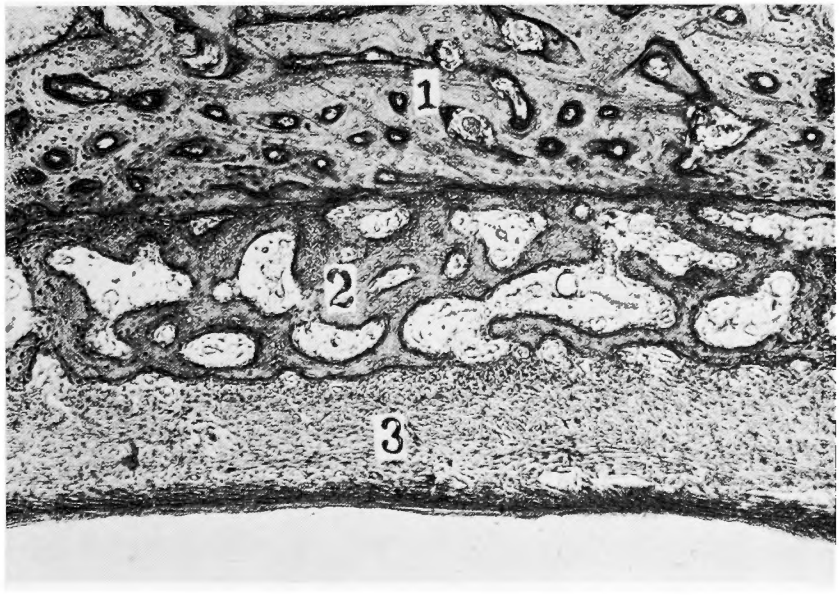

Fig. 22 A part of Fig. 21 (6) is enlarged (× 55). (1) Graft-bone, its lacunae are empty. (2) Network-like new bone, probably produced by meninx (3) which is remarkably thickened.

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（和文抄録）

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江本修治

煮沸骨と新鮮骨を同一犬の頭蓋骨と腓骨とに移植して1年後の状態を比較観察した．

1. 腓骨，頭蓋骨共に1年後には改造行程は終止状態にあつた．

2. 1年後の腓骨：新鮮骨，煮沸骨共に改造著しく骨性癒合を営み，肉眼的にもレントゲン学的にも移植個所の不明なものが多かつた．組織学的にも骨細胞，ハーバース管等は正常の状態にあるが，死滅骨小体が集合して斑状をなす部分がある．この骨死滅斑は改造されずに残つた移植骨の部分であるが，煮沸骨の死滅斑は新鮮骨のそれより大きい．

3. 1年後の頭蓋骨：新鮮骨，煮沸骨共に部分的骨癒合の程度のものが多く，レントゲン写真によると移植部はすべての標本に於て明瞭であつた．組織学的に見ると移植新鮮骨では広範囲に亘つて新骨を以て置換されており，小さい死滅斑が島状に残つているに過ぎない．これに反して移植煮沸骨では死滅斑が大きく，骨表面，ハーバース管，髄腔等の周囲にて小範囲に限り改造されているものが多い．

4. 1年後の標本では自家新鮮骨と同種新鮮骨の間には顕著な差異がなく，又自家煮沸骨と同種煮沸骨の間にも著しい差は認められなかつた．

5. 移植新鮮骨自身の改造は移植煮沸骨の改造より早期に起り且つ早く完結する．

6. 骨細胞はすべての移植骨に於て一度消失するが，新鮮骨の骨細胞消失は煮沸骨のそれよりも早期に起る．

7. 頭蓋骨の骨新生には頭蓋骨膜と脳硬膜が関与する．

8. 骨新生に際しては，腓骨には軟骨組織の出現をみるが，頭蓋骨には出現しない．